2. Literature Review

Biswas et al., (2010) developed high performance liquid chromatography method for determination of paracetamol, chlorzaxozone and diclofenac potassium. The chromatographic method was standardized using a reverse phase C-18 column with UV detector at 254 nm. Mobile phase consisted of methanol and (0.01M) monobasic sodium phosphate (70:30) (pH was adjusted to 2.5 ± 0.2 using 10% orthophosphoric acid) and the flow rate was 1 ml/min. The recovery values were between 99.65% and 101.20%. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The method was successfully applied for the determination of paracetamol, chlorzaxozone and diclofenac potassium in marketed formulations.

Freddy et al., (2010) carried out a simple, specific, accurate and economical isocratic reverse phase liquid chromatographic (RP-HPLC) method for the determination of paracetamol, acetyl salicylic acid, mefenamic acid and cetirizine dihydrochloride. Separation was achieved with a Nucleodur, C–18 column having 250 x 4.6 mm i.d. with 5 μm particle size. Disodium hydrogen phosphate (pH-6.5) and acetonitrile (60:40 v/v) was used as eluent at a constant flow rate of 1.0 ml/min. UV detection was performed at 220 nm. The retention times of acetyl salicylic acid, paracetamol, mefenamic acid and cetirizine dihydrochloride were found to be 2.01 min, 2.92 min, 4.91 min and 10.2 min respectively. The proposed method was validated and successfully used for estimation of paracetamol, acetyl salicylic acid, mefenamic acid and cetirizine dihydrochloride in the pharmaceutical dosage forms.

Kasawar et al., (2010) developed and validated stability indicating RP-HPLC method for simultaneous estimation of related substances of albuterol sulfate and ipratropium bromide in nasal solution. Heat, acid, base, UV radiation and oxidation stress conditions were applied to check the stability of combined dosage form. The chromatographic conditions were optimized using an inertsil C-8 column with dimensions 250 mm x 4.6 mm, 5 μm. Mobile phase was consisted of solvent A (solution containing 2.5 g of potassium di hydrogen phosphate and 2.87 gm of heptane-1-sulfonic acid sodium salt per liter of water, adjusted to pH 4.0 with ortho phosphoric acid) and solvent B
(acetonitrile). Flow rate was maintained 1.0 ml min\(^{-1}\). The analytes were detected and quantified at 210 nm using photodiode array (PDA) detector\(^3\).

**Kumudhavalli et al., (2010)** developed a rapid, sensitive and specific RP-HPLC method using UV detection for determination and quantification of pseudoephedrine hydrochloride, cetirizine dihydrochloride and paracetamol and validated the same. Optimization of chromatographic conditions was carried out on a pre-packed Crosmosil C-8 (250x4.6 mm) column using filtered and degassed mixture of buffer and acetonitrile in the ratio of 85:15 as mobile phase at a flow rate of 1.0 ml/min. Effluent was monitored at 215 nm. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness and specificity\(^4\).

**Nora et al., (2010)** developed RP-HPLC method for the simultaneous determination of phenylephrine hydrochloride and chlorpheniramine maleate using UV spectrophotometry. A reversed-phase column and mobile phase of methanol : water : acetonitrile (80:12:8 v/v/v) at 0.9 ml/min flow rate was used to separate both drugs with a UV detection at 270 nm. The limit of detection was found to be 0.142 and 0.342 µg/ml for phenylephrine hydrochloride and chlorpheniramine maleate. The limit of quantification was noted to be 0.550 and 0.715µg/ml for phenylephrine hydrochloride and chlorpheniramine maleate, respectively. Recovery values were between 99.79-101.49%. They concluded that the proposed method was precise, accurate, selective and rapid for the simultaneous determination of phenylephrine hydrochloride and chlorpheniramine maleate. The results obtained using the proposed methods were statistically analyzed\(^5\).

**Fegade et al., (2009)** carried out simultaneous estimation of paracetamol and piroxicam in tablets. Separation was achieved on Eurosphere 100 C-18 as stationary phase. Mobile phase was methanol: water (70:30). Detection wavelength was 227nm. Recovery values were between 99.44 and 100.01%. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH and USP guidelines. The method was successfully applied for the determination of paracetamol and piroxicam in marketed formulations\(^6\).
**Godse et al., (2009)** reported a reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. An ODS C$_{18}$ (Intersile 25 cm x 4.6 mm, 10µm) column was used as stationary phase. Mobile phase used was methanol: water (70:30 v/v) at flow rate of 1mL/min. Linearity was observed in the concentration range of 2-50µg/ml for aceclofenac and 5-50µg/ml for paracetamol. Recoveries values of aceclofenac and paracetamol were 100.6% and 100.7% respectively. They concluded that the proposed method was precise, accurate, selective and rapid for the simultaneous determination of aceclofenac and paracetamol\(^7\).

**Hadad et al., (2009)** worked on development and validation of stability- indicating RP-HPLC method for determination of paracetmol with dantrolene or/and cetirizine and pseudoephedrine in pharmaceutical dosage forms. Stability indicating capability of the method was demonstrated by adequate separation of these four analytes from all the degradants peaks. A gradient mobile phase system consisting of acetonitrile and 50 mmol/lit sodium dihydrogen phosphate, 5 mmol/lit heptane sulfonic acid sodium salt was used. Separation was achieved by HS C$_{18}$ analytical column. Quantitation was monitored on UV detector at 214 nm. The developed method was sensitive, accurate and precise\(^8\).

**Karthikeyan et al., (2009)** developed HPLC method for the determination of paracetamol, chlorzoxazone and aceclofenac in pharmaceutical formulation. Separation was achieved by Phenomenex C$_{18}$ column using a mixture of acetonitrile: 0.05 M disodium hydrogen orthophosphate (65:35) (pH 3.0) as a mobile phase. UV detection wavelength was 271 nm. The calibration curves were linear in the range of 20-100µg/ml for paracetamol, chlorzoxazone and 4-20 µg/ml for aceclofenac. LOD was found to be 0.9µg/ml, 1.81µg/ml and 0.9µg/ml for paracetamol, chlorzoxazone and aceclofenac respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines\(^9\).

**Louhaichi et al., (2009)** determined pseudoephedrine, pheniramine, guaifenesin, pyrilamine, chlorpheniramine and dextromethorphan in cough and cold medicines using HPLC. The separation was achieved by Kromasil C$_{18}$ column as a stationary phase.
Mobile phase was methanol: di-hydrogen phosphate buffer (pH 3.0) (45:55). The analyses were performed at flow rate 1ml/min and detection wavelength was 220 nm. Range was 5-60μg/ml for pseudoephdrine, pheniramine, chlorpheniramine and 50-600μg/ml for pyrilamine, dextromethorphan. The recovery values were between 98.5 and 101.71%\(^{10}\).

**Raman et al., (2009)** validated stability-indicating RP-HPLC method for famciclovir. The method used Intersil ODS 3 V (250 x 4.6mm, 5μm) column as a stationary phase. The mobile phase used was mixture of 0.01 M potassium dihydrogen orthophosphate buffer (pH 3.0) and methanol (80:20). The degradation products were well resolved from standard peak and its impurities. They concluded that famciclovir degraded significantly in oxidative, acid and base conditions and mildly in hydrolytic conditions. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines\(^{11}\).

**Bhatia et al., (2008)** reported reverse phase high performance liquid chromatography and spectrophotometric method for the estimation of ambroxol hydrochloride and cetirizine hydrochloride in combined dosage form. The chromatographic methods were optimized using a (HIQSIL) C-18 column with dimensions 250 X 4.6 mm, i.d. 10 μm particle size, with UV detection at 229 nm. Mobile phase used was mixture of methanol, acetonitrile and water at fixed ratio of 40:40:20 v/v. The linearity range was 5–55μg/ml and 10–40μg/ml for ambroxol hydrochloride and cetirizine hydrochloride respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines \(^{12}\).

**Crevar et al., (2008)** carried out RP-HPLC analysis of caffeine, paracetamol and its degradation product p-aminophenol. Mobile phase was consisted of methanol and phosphate buffer (20:80 v/v). The flow rate was 1 mL/min at 230nm. Separation was achieved using Zorbax Extend C-18 Column (150 mm × 4.6 mm, 5 μm). The proposed method was simple, accurate, economical, rapid and reproducible. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The results obtained in this study were precise, rapid and sensitive\(^{13}\).
Karakus et al., (2008) developed and validated a rapid reverse phase high performance liquid chromatography method for the determination of cetirizine or fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms. Separation was achieved by C-18 column with mobile phase acetonitrile and phosphate buffer (60:40 v/v). The wavelength was 230 nm. The recovery was more than 99%. The method was simple, precise, rapid and reliable. The proposed method gave a good resolution between cetirizine, fexofenadine and pseudoephedrine.

Nagappan et al., (2008) developed an RP-HPLC method for simultaneous estimation of ambroxol hydrochloride and loratidine in pharmaceutical formulation. The method was carried out on a Phenomenex Gemini C18 (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: 50 mM ammonium acetate (50:50 v/v) at a flow rate of 1.0 ml/min. Detection was done at wavelength 255 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines.

Sawsan et al., (2008) reported simultaneous determination of phenylephrine hydrochloride, guaifenesin, and chlorpheniramine maleate in cough syrup by gradient liquid chromatography. Separation was achieved by C-8 column using 0.005 M heptane sulfonic acid sodium salt (pH 3.4) and acetonitrile as a mobile phase by gradient elution at different flow rates. The separated drugs were detected using UV detector at wavelength 210 nm. The responses were linear in the concentration range of 30-180μg/ml, 120-1800μg/ml and 10-60μg/ml for phenylephrine hydrochloride, guaifenesin, and chlorpheniramine maleate respectively.

Shirkhedkar et al., (2008) carried out simultaneous determination of paracetamol and piroxicam in tablets by HPLC combined with densitometry forms using a mixture of n-dichloroethane, methanol and triethylamine (30:20:50 v/v) as mobile phase. The wavelength was 284 nm with flow rate 1 mL/min. The developed HPLC method was simple, precise, accurate and reproducible and can be used for simultaneous determination of paracetamol and piroxicam in tablets.
Shaikh et al., (2008) carried out HPLC analysis for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form. Chromatographic separation was achieved on Xterra RP-18 (250mm×4.6mm, 5µm) analytical column. A mixture of acetonitrile: di potassium phosphate (30mM pH 9.0) in ratio 50:50, v/v at a flow rate of 1.7 ml/min was used as mobile phase. The detection wavelength was 215 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The method was successfully applied for simultaneous determination of ambroxol hydrochloride and azithromycin in marketed formulations.

Palabıyık et al., (2007) carried out HPLC analysis of phenylephrine hydrochloride, paracetamol, chlorpheniramine maleate and dextromethorphan hydrobromide in pharmaceutical preparations. The separation was achieved by C18 column using a gradient mobile phase of acetonitrile: sodium perchlorate (pH 3, 0.01 M) at a flow rate of 1.4 ml/min. Detection wavelength was 204 nm. LOQ for phenylephrine hydrochloride, paracetamol, chlorpheniramine maleate and dextromethorphan hydrobromide were 0.08µg/ml, 0.29µg/ml, 0.24µg/ml and 0.03 respectively. LOD for the phenylephrine hydrochloride, paracetamol, chlorpheniramine maleate and dextromethorphan hydrobromide was 0.03 µg/ml, 0.10µg/ml, 0.08µg/ml and 0.13µg/ml respectively. The method was suitable for the determination of the four compounds in sugar-coated tablet.

Bhavsar et al., (2006) estimated paracetamol and valdecoxib in combined dosage form by RP-HPLC. Separation was achieved by Luna C18 column and methanol: phosphate buffer (pH 3.5) (60: 40) as eluent. Detection wavelength was 242 nm. Etoricoxib was used as an internal standard. Concentration range for paracetamol and valdecoxib was 25-150µg/ml and 1-6µg/ml respectively. The average recovery of paracetamol and valdecoxib was 101.1% and 101.7% respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The developed method was accurate, precise and selective.
**Grossa et al., (2006)** developed a simultaneous stability indicating, HPLC-DAD method for determination of guaifenesin, methyl and propyl-parabens in cough syrup. The isocratic separation and quantitation were achieved within 17 min on 150 mm column with an ether-linked phenyl stationary phase and a hydrophilic end capping. The mobile phase was consisted of eluent A: 10 mM aqueous phosphate buffer (pH 3.0) and acetonitrile in ratio 25:75 (v/v) and eluent B as methanol. The eluent A and B were kept in ratio 85:15 (v/v) with a flow rate 1ml min\(^{-1}\). The detection of analytes was carried out at dual wavelength as 254 and 276 nm. Heat, acid, base, UV radiation and oxidation stress conditions were performed for stability of combined dosage form\(^{21}\).

**Kambia et al., (2006)** determined stability and compatibility of paracetamol injection with ketoprofen using HPLC. The mobile phase used was methanol and phosphate buffer (65:35v/v) at 230 nm wavelength. The paracetamol and ketoprofen were physically compatible and chemically stable upto 48 hrs at room temperature. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines\(^{22}\).

**Schieffer et al., (2006)** developed a method for simultaneous estimation of phenylepherine hydrochloride, phenylpropanolamine hydrochloride and guaifenesin in dosage forms by reverse phase paired-ion high performance liquid chromatography. Heat, acid, base, UV radiation and oxidation stress conditions were applied to study the stability of combined dosage form. The separation was carried out on a Waters Bondapak C18 column (300 x 3.9 mm). The eluent was 5 mM hexanesulfonic acid sodium salt in 1% glacial acetic acid and methanol in ratio of 80/20 (v/v). Wavelength for detection of drugs was kept at 220 nm\(^{23}\).

**Selvan et al., (2006)** reported simultaneous estimation of ambroxol, phenylpropanolamine, levocetirizine and paracetamol in combined dosage forms by reversed-phase high performance liquid chromatography. The separation was carried out by Hichrom C\(_{18}\) column with mobile phase acetonitrile: buffer: triethylamine (30:70:0.5). Detection was carried out at 238nm. Tadalafil was used as an internal standard. The LOD values for levocetirizine, ambroxol, phenylpropanolamine and
paracetamol were 5ng/ml, 100ng/ml, 200ng/ml and 15ng/ml and LOQ were 15ng/ml, 100ng/ml, 200ng/ml and 15ng/ml respectively. The recovery values were between 99.58 and 100.00%.

Sun et al., (2006) reported simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in pharmaceutical dosage forms. Separation and quantitation were achieved by C<sub>18</sub> column. The mobile phase was methanol: phosphate buffer (45:55), containing 0.1% triethylamine and pH was adjusted 3.6 with phosphoric acid. All drugs were detected by UV detector at 260 nm. The recovery values were between 99.25% and 101.98%. The method was simple, sensitive and could be used in estimation of commercially available formulation.

Tan et al., (2006) carried out simultaneous determination of pseudoephedrine and cetirizine in human plasma using liquid-chromatography–ion trap spectrometry by Hypersil C-18 column with methanol and 1mM ammonium acetate (65:35v/v) as mobile phase at 230 nm by UV detector. Bio-analytical study was specific, sensitive, accurate and precise. The method was robust and can be successfully applied to pharmacokinetics study of pseudoephedrine and cetirizine in human plasma.

Olmo et al., (2005) developed new approaches with cyano columns for the separation of acetaminophen, phenylephrine, chlorpheniramine and related compounds. Separation was achieved by discovery cyanopropyl (SUPELCO) column. Mobile phase was aqueous: organic solvent (95:5, v/v). The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness. Recovery values were observed between 102% and 108%.

Barbas et al., (2004) developed a stability indicating HPLC method for determination of dextromethorphan and guaifenesin in cough syrup. Forced degradation study was also carried out to check the stability of the syrup. Mobile phase used was phosphate buffer (25 mM, pH 2.8) with triethylamine (TEA): acetonitrile (75:25, v/v). Stress conditions like heat, acid, base, UV radiation and oxidations were applied to study the stability of combined dosage form.
Ferrayolli et al., (2004) carried out validation of a chiral HPLC assay for (R)-salbutamol sulfate. The method was validated in terms of specificity, robustness, linearity, precision and accuracy. Under the chromatographic conditions of the method, known impurities were separated from the active principle. Separation was achieved by 250 mm × 4.6 mm Chirobiotic T column (amphoteric glycopeptide teicoplanin bonded to a 5 µm silica gel). A mixture with fixed ratio of acetonitrile, methanol, acetic acid, triethylamine in the ratio 60:40:0.3:0.2 (v/v/v/v) was used as mobile phase. Flow rate was kept at 1.5 ml min⁻¹. The drugs were monitored at 276 nm wavelength.

Marin et al., (2004) carried out LC/MS analysis for the degradation profiling of cough-cold product under forced conditions. Heat, acid, base, UV radiation and oxidation stress conditions were applied to study the stability of cough–cold products. Liquid chromatography coupled with mass spectrometry was used to analyze the degraded samples and obtain molecular weights information. Chromatographic analysis was performed on a 5 µm particle, Discovery HS PEG column Supelco 15 cm x 0.46 cm at 35°C. Mobile phase was phosphate buffer 20 mM (pH 7.0) : acetonitrile 90:10 v/v. Flow rate was 1.0 ml/min with UV detection at 215 and 254 nm.

Basavaraj et al., (2003) reported HPLC method for simultaneous estimation of paracetamol and cetirizine hydrochloride in tablet dosage form by C-18 column with mobile phase acetonitrile and water (40:60 v/v). The UV detection wavelength was 230 nm. The retention time was 2.29, 3.5 and 5.88 minutes for paracetamol, cetirizine hydrochloride and nimesulide, respectively. The % recovery was between 99.68 and 100.13. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The proposed method was simple, accurate, economical, rapid and reproducible.

Garcia et al., (2003) developed HPLC method for determination of paracetamol, phenylephrine hydrochloride and chlorpheniramine maleate in capsule using HS PEG C-18 column with mobile phase consisting of 20 mM phosphate buffer and acetonitrile (65:35v/v). The UV detection was performed at 215 nm for all compounds. The method
was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The recovery values were between 99.25% and 101.98%. The method was simple, sensitive and could be used in estimation of commercially available formulations\textsuperscript{32}.

**Goger et al., (2003)** reported UV spectrophotometric and high performance liquid chromatographic method for quantitative determination of ambroxol in tablet dosage form. A reversed-phase C-18 column with mobile phase consisting of aqueous phosphate (0.01 M), acetonitrile, glacial acetic acid (59:40:1, v/v/v) (pH 3.12) was used. UV detection was done at 252 nm. The limit of detection was noted to be 0.278 and limit of quantification was 0.705 μg/ml. Recovery was noted to be between 99.08 and 101.26%. The method was evaluated for specificity, robustness, linearity, precision and accuracy\textsuperscript{33}.

**Hood et al., (2003)** carried out simultaneous analysis of codeine phosphate, ephedrine HCl and chlorpheniramine maleate in cough syrup formulation. They used Eurosphere 100 C\textsubscript{18} column as stationary phase. Mobile phase was methanol: water (70:30). Detection wavelength was 227 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The method was applied for the analysis of these analytes in commercially available tablets\textsuperscript{34}.

**Kim et al., (2003)** developed a simple method for determination of ambroxol in human plasma using LC-MS/MS. The stationary phase was C-18 X-Terra MS column (2.1cm X 30 mm) with 3.5 μm particle size. The mobile phase was composed of 20 mM ammonium acetate in 90% acetonitrile (pH 8.8). The flow rate was 2.50 ml/min. The recovery values were between 100.35% and 101.28%. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The proposed method was simple, accurate, economical, rapid and reproducible\textsuperscript{35}.

**Nagaralli et al., (2003)** worked on liquid chromatographic determination of cetirizine hydrochloride and paracetamol in human plasma and pharmaceutical formulations.
Separation was achieved by CLC C18 (5μ × 25cm×4.6mm i.d) column as a stationary phase. Detection wavelength was 230 nm. The mobile phase was acetonitrile: water (55:45 v/v). The linearity range was 0.715–55μg/ml and 0.55–39μg/ml for cetirizine and paracetamol, respectively. The limits of detection were 0.248 and 0.208μg/ml and limits of quantification were 0.550 and 0.715μg/ml for cetirizine and paracetamol respectively. Recovery values were between 99.28 and 100.19%. The method was applied for the analysis of these analytes in commercially available formulations36.

Qi et al., (2003) carried out HPLC analysis of paracetamol, caffeine, chlorpheniramine maleate and guaiacol glyceride ether in syrup by Diamonsil C-18 column with mobile phase consisting of methanol and acetic acid (55:45v/v) at 215 nm. The retention times were noted to be 4.8, 6.3, 7.5 and 9.5 minutes respectively for chlorpheniramine maleate, paracetamol, caffeine and guaiacol glyceride ether. The recovery values were between 99.97% and 100.95%. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The proposed method was simple, accurate, economical, rapid and reproducible. The method was sensitive and could be used in estimation of commercially available formulations37.

Wang et al., (2003) developed HPLC method for analysis of paracetamol, caffeine and chlorpheniramine maleate in tablet using C-18 column with mobile phase 5 mM aq. solution of hexane sulfphonic acid, 10 mM tri ethyl amine and 1% acetic acid and (20:30:30v/v) and detection at 223 nm. The proposed method was simple, accurate, economical, rapid and reproducible. The limits of detection were 0.532, 0.765 and 0.534 μg/ml and limits of quantification were 0.987, 0.875 and 0.782 μg/ml for paracetamol, caffeine and chlorpheniramine maleate, respectively. Recovery values were between 100.08 and 101.19%. The method was successfully applied for simultaneous determination of paracetamol, caffeine and chlorpheniramine maleate in marketed formulations38.

Marin et al., (2002) developed a new HPLC method for quantification of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations,
capsules and sachets using C-18 column. The mobile phase was mixture of 40 mM phosphate buffer and acetonitrile (75:25 v/v). The detection was monitored at 238 nm. The method was precise and accurate for determining concentrations in the range 0.15 to 0.46 mg/ml for acetaminophen, 0.003 to 0.009 mg/ml for phenylephrine and 0.001 to 0.004 mg/ml for chlorpheniramine.

Panda et al., (2002) reported simultaneous analysis of phenylpropanolamine, chlorpheniramine and bromhexine in syrup by derivative spectrophotometry. The analysis was done on spherisorb C8 (4.6 × 250MM, 5 µm) column. The mobile phase was acetonitrile and sodium dihydrogen orthophosphate (75:25). The flow rate was 1.5 mL/min. The detection wavelength was 224 nm. The proposed method was simple, accurate, economical, rapid and reproducible.

Qi et al., (2002) carried out simple HPLC method for simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in tablet formulations. The mobile phase was composed of 20 mM ammonium acetate in 60% acetonitrile (pH 4.5) at a flow rate 1.5 ml/min. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. Linearity, accuracy and precision were found to be acceptable over the ranges 31.6-315.8µg/ml for acetaminophen, 9.5-94.6µg/ml for caffeine and 1.4-13.8µg/ml for chlorpheniramine maleate. Recovery values were between 98.3 and 101.5%.

Senyuva et al., (2002) developed and validated high-performance liquid chromatographic method for determination of paracetamol, phenylephrine HCl, and chlorpheniramine maleate in pharmaceutical dosage forms. Separation was achieved using Bondapak CN RP analytical column (125 Å, 10 µm, 3.9 × 150 mm) as stationary phase. The mobile phase was mixture of acetonitrile and phosphate buffer (pH 6.22, 78:22). LOD for phenylephrine and chlorpheniramine was 0.0325µg/ml, 0.0272µg/ml. LOQ was 0.251µg/ml and 0.184 for phenylephrine and chlorpheniramine respectively. The recovery values were between 98.0 and 99.73%. It was concluded that method was reliable and reproducible for the determination of the active ingredients in pediatric cough–cold syrups.
Takagaki et al., (2002) developed and validated simple and sensitive method for determination of chlorpheniramine maleate in human plasma using liquid chromatography–mass spectrometry. The mobile phase used was water and acetonitrile (60:40v/v) and detection was done at 238 nm wavelength. The developed LC–MS–MS method was convenient and sensitive. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The method was applied for the analysis of these analytes in commercially available formulations⁴³.

Heinanen et al., (2001) carried out HPLC analysis for quantification of ambroxol hydrochloride and benzoic acid in syrup dosage form for stability evaluation. Chromatographic conditions for the method included; stationary phase symmetry shield RP C₈, 5 mm, 250 mm X 4.6 mm, and mobile phase methanol : H₃PO₄ (8.5 mM pH 2.8 adjusted with triethyl amine) in ratio 40:60 (v/v) at 247 nm. The recovery values were between 99.88% and 101.17%. The method was simple, sensitive and could be used in estimation of commercially available formulations⁴⁴.

Kartal et al., (2001) carried out LC method for the analysis of paracetamol, caffeine and codeine phosphate in pharmaceutical preparations. Separation was achieved using Bondapack C₈ column with flow rate 1.0 ml/min. The mobile phase composition was phosphate buffer, methanol, acetonitrile, isopropyl alcohol in the ratio of 20/20/30/30 (v/v/v/v). Detection was carried out at 215 nm. Linearity ranges were 0.400-1500μg/ml, 0.075-0.90μg/ml and 0.300-30μg/ml for paracetamol, caffeine and codeine phosphate respectively. While the limits of detection were 0.150, 0.023 and 0.10μg/ml. Limits of quantification were 0.400, 0.075 and 0.300μg/ml for paracetamol, caffeine and codeine phosphate, respectively. Recovery values were between 94.40 and 106.09%. Method was validated in terms of linearity, reproducibility, specificity, sensitivity and ruggedness⁴⁵.

Celma et al., (2000) carried out simultaneous determination of paracetamol and chlorpheniramine in human plasma by liquid chromatography–tandem mass spectrometry. The mobile phase was composed of water and acetonitrile at flow rate 1.2
ml/min. The limits of detection were 0.15, and 0.32 μg/ml and limits of quantification were 0.76, and 0.98 μg/ml for paracetamol and chlorpheniramine, respectively. Recovery values were between 99.40 and 101.09%. LC–MS–MS method was accurate, precise and reliable for estimation of paracetamol and chlorpheniramine concentrations in human plasma after oral administration.

**Koundourellis et al., (2000)** developed a high performance chromatographic method for estimation of ambroxol in presence of different preservatives in pharmaceutical preparations. The eluents were monitored by UV detector at 247 nm. The mobile phase used was 0.05 M ammonium acetate buffer (pH 3.45) with glacial acid and methanol in ratio 30:70 (v/v). The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The developed method was simple, accurate, economical, rapid and reproducible.

**Muszalska et al., (2000)** worked on HPLC analysis of paracetamol, caffeine, ascorbic acid and phenylephrine hydrochloride in tablet formulations using C-18 column with mobile phase 0.015 phosphate buffer and methanol (65:35v/v) at 254 nm. The flow rate was maintained at 1.2 ml/min. The method was simple, selective, rapid, accurate and precise for simultaneous determination of these drugs. The recovery values were between 99.25% and 101.98%. The method was simple, sensitive and could be used in estimation of commercially available formulations.

**Sahu et al., (2000)** carried out spectrophotometric analysis for simultaneous determination of chlorpheniramine maleate, phenylpropanolamine HCl and dextromethorphan hydrobromide in syrup dosage forms. The wavelength maxima of phenylpropanolamine and chlorpheniramine maleate and bromhexine hydrochloride in 0.1 N hydrochloric acid were found to be 257, 265 and 278nm respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The drugs obeyed Beer’s law in the concentration ranges of 200-600μg/ml, 10-32μg/ml and 50-160μg/ml for
phenylpropanolamine, chlorpheniramine and dextromethorphan respectively. The recovery values were between 98.69% and 100.80%\textsuperscript{49}.

\textbf{Stewart et al., (2000)} carried out HPLC analysis for determination of guaifenesin with selected medications on underivatized silica with an aqueous-organic mobile phase. The separation and quantitation was achieved on a 25 cm underivatized silica column using a mobile phase of (60:40 v/v) 6.25 mM phosphate buffer (pH 3.0) and acetonitrile at a flow rate of 1 ml/min. UV detection of all analytes were done at 216 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The developed method was simple, accurate, economical, rapid and reproducible.\textsuperscript{50}.

\textbf{Vasudevan et al., (2000)} developed a liquid chromatographic method for simultaneous estimation of phenylpropanolamine HCl, guaifenesin and diphenylpyraline HCl in syrup dosage form. The stationary phase used was Shimpak® C8 column with mobile phase acetonitrile, triethylamine buffer (pH adjusted to 3.5 using orthophosphoric acid; 0.5%), in ratio 35:65 (v/v). The flow rate was maintained at 1.2 ml/min. Detection was carried out at wavelength 210 nm. Recovery values were between 99.50 and 101.09%. The method was successfully applied for simultaneous determination of phenylpropanolamine HCl, guaifenesin and diphenylpyraline HCl in marketed formulations\textsuperscript{51}.

\textbf{Suzen et al., (1999)} reported high performance liquid chromatographic method for simultaneous estimation of guaifenesin and codeine phosphate in tablets. The separation achieved on a Kromasil C-18 column. Isocratic mobile phase containing affixed ratio of methanol–di hydrogen phosphate buffer at pH 3.0 (45:55, v/v) was used. The analysis was performed at a flow rate of 1.0 ml min\textsuperscript{-1} and detection wavelength was 220 nm. The method was validated in terms of specificity, robustness, linearity, precision and accuracy. The limits of detection were 0.33 and 0.41 μg/ml for guaifenesin and codeine phosphate and limits of quantification were 0.550 and 0.715μg/ml for guaifenesin and codeine phosphate, respectively. The recovery values were between 98.95% and 100.88%\textsuperscript{52}. 

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Erka et al., (1998) reported simultaneous high performance liquid chromatographic and derivative ratio spectra spectrophotometry determination of chlorpheniramine maleate and phenylephrine hydrochloride by Li-Chrosorb C-18 column with mobile phase methanol and phosphate buffer (70:30 v/v). The flow rate was maintained at 1.0 ml /min. Detection was carried out at wavelength 267nm. Recovery values were between 100.01 and 100.92%. The method was successfully applied for simultaneous determination of chlorpheniramine maleate and phenylephrine hydrochloride in marketed formulations.

Basley et al., (1997) investigated degradation of salbutamol after gamma irradiation using HPLC and ESR spectroscopy. The separation was carried out on a Waters - Bondapak C-18 column (300 x 3.9 mm). The eluent was 5 mM hexane sulfonic acid sodium salt in 1% glacial acetic acid (aqueous) and methanol in a ratio of 80/20 (v/v). The UV detection wavelength was 220 nm and flow rate was 1 ml/min. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The developed method was simple, accurate, economical, rapid and reproducible.

Barnal et al., (1996) compared a high performance liquid chromatography with superficial fluid chromatography for determination of salbutamol sulphate and its impurities in pharmaceutical formulations. For optimum separation of active compounds, gradient elution using aqueous buffer pH 3.0 and acetonitrile was undertaken. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness. The method was simple, sensitive and could be used in estimation of commercially available formulations.

Yamato et al., (1996) carried out simultaneous determination of chlorpheniramine maleate by high-performance liquid chromatography using tetra-n-butyl ammonium phosphate as an ion-pair reagent. Separation was achieved by Capcell Pak C-8 column. Isocratic mobile phase consisting of 50 mM KH₂PO₄, tetra-n-butyl ammonium phosphate as an ion-pair reagent and methanol in the ratio of 80:2:18 v/v was used. The UV detection was done at wavelength of 245 nm. The limit of detection was noted to be
0.31 μg/ml for chlorpheniramine maleate while the limit of quantification was 0.71 μg/ml. The method was simple and sensitive.\(^{56}\)

**Indrayanto et al., (1995)** performed simultaneous assay of phenylpropanolamine HCl, caffeine, paracetamol, glyceralguaiacolate and chlorpheniramine maleate in silabat tablets using HPLC coupled with diode array detector. Analysis was carried out by LiChrospher 100CN as stationary phase. Mobile phase was acetonitrile: tetrahydrofuran: ion pair solution (7:6:87 v/v) (pH was adjusted at 3.3) at flow rate 1 ml/min. Detection wavelengths were 265nm, 260nm, 298nm, 284nm and 310nm for phenylpropanolamine HCl, chlorpheniramine maleate, caffeine, glyceralguaiacolate and paracetamol respectively. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness. The recovery values were between 98.5 and 101.5%.\(^{57}\)

**Laine et al., (1995)** carried out HPLC analysis to study decomposition of salbutamol in aqueous solution. They attempted to demonstrate the effect of different buffer concentration, buffer species, role of pH, and antioxidants on stability of formulation. A Li-Chrosorb reverse phase C-18 column (125 × 4 mm i.d., particle size 5 µm) was used as stationary phase. The mobile phase consisted of acetonitrile, sodium dihydrogen phosphate (40 mM) and triethylamine (5.74 mM). The flow rate was 1.5 ml/min at wavelength 220 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness. The developed method could be used in estimation of commercially available formulations.\(^{58}\)

**Yamaguchi et al., (1994)** carried out high-performance liquid chromatographic determination of phenylephrine in human serum. Separation was achieved by Intersil ODS C-18 column with fluorescence spectrometer. Mobile phase was potassium dihydrogen phosphate and methanol in the ratio of 60:40 (v/v). Linearity range was 5-500ng/ml. The recovery values were between 88.0 and 95.5%. The method was applied for the determination of phenylephrine in human serum after oral administration of phenylephrine hydrochloride.\(^{59}\)
Shanawany et al., (1990) worked on HPLC analysis of paracetamol, caffeine, ascorbic acid and phenylephrine hydrochloride in tablet formulation. Separation was achieved using Eurosphere 100 C-18 column, in isocratic mode. Mobile phase was methanol: water (70:30 v/v). The flow rate was maintained at 1.0 ml/min. The detection wavelength was 227 nm. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness as per ICH guidelines. The developed method was simple, selective, rapid, accurate and precise. The recovery values were between 100.11% and 101.33%.

Gupta et al., (1984) worked on quantitation of acetaminophen, chlorpheniramine maleate, dextromethorphan HBr and phenylpropanolamine HCl in combination using HPLC. The column Bondapak phenyl (30cm X 4mm i.d.) with three different mobile phases was used for adequate separation of drugs at flow rate of 1 ml/min. Detection wavelength was 256nm. Recovery values were between 98.7-101.4%. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation, robustness and ruggedness. The developed method was simple, accurate, economical, rapid and reproducible. The method was successfully applied for simultaneous determination of acetaminophen, chlorpheniramine maleate, dextromethorphan HBr and phenylpropanolamine HCl in marketed formulations.
2.1. Research Envisaged

Pharmaceutical analysis plays a very significant role in quality control of pharmaceutical products through a rigid check on raw materials, in process samples and finished products. It is instrumental in ensuring the quality of pharmaceutical products and requires development of methods with high degree of accuracy and precision. The accuracy and precision depend upon the relative and absolute errors. Errors will be minimized if the method is simple. Thus simplicity of the method can be indirectly related to accuracy and precision. Therefore it is one of the prime considerations while developing analytical method.

Literature survey revealed that as such no HPLC method has yet been reported for simultaneous estimation of the drugs selected for the present study. The drugs include Ambroxol hydrochloride, Cetirizine hydrochloride, Chlorpheniramine maleate, Guaiphenesin, Paracetamol, Phenylephrine hydrochloride and Salbutamol sulphate.

The nonavailability of HPLC method for the analysis of the combination of above mentioned drugs made it worth while to pursue the present research work. The objective of the present work was to develop simple, accurate, precise and rapid stability indicating analytical methods for simultaneous estimation of the selected drugs in marketed dosage forms.

The developed method was also validated as per ICH guidelines. Assay validation assures the accuracy and reliability of test results for drug identity, strength, quality and purity. A validated analytical method is often employed for product testing at various critical stages of a manufacturing to check and ensure whether the manufacturing process does what it purports to do.
2.2. Plan of Work

The work was carried out on the following lines:

- Exhaustive literature survey
- Selection of drug candidates
- **Method development**
  - Procurement of drugs sample, chemicals and solvents
  - Selection of solvent system
  - Wavelength selection
  - Chromatographic conditions
    (Column, mobile phase, pH, temperature, concentration range, modifier etc.)
- **Method validation with respect to parameters such as:**
  - Linearity
  - Range
  - Specificity
  - Accuracy
  - Precision
  - Limit of detection
  - Limit of quantitation
  - Statistical validation
- Estimation of selected drugs in marketed formulations
- Stability studies
- Compilation and submission of the thesis
References


